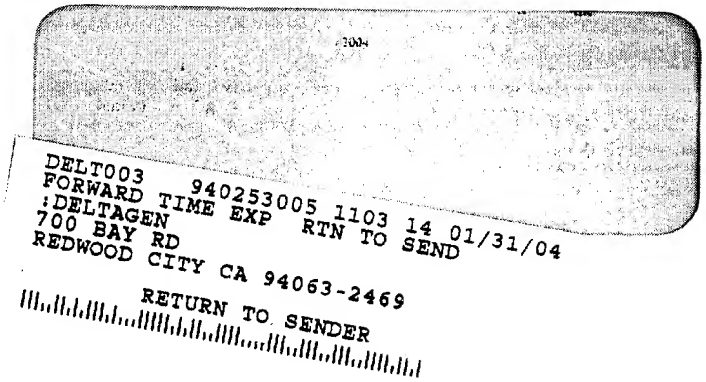
TC 1600 CMI
Organization_____ Bldg./Room_____
U. S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
WASHINGTON, DC 20231
IF UNDELIVERABLE RETURN IN TEN DAYS

OFFICIAL BUSINESS

AN EQUAL OPPORTUNITY EMPLOYER

COPY OF PAPERS
ORIGINALLY FILED


DELT003 940253005 1103 14 01/31/04
FORWARD TIME EXP RTN TO SEND
:DELTAGEN
700 BAY RD
REDWOOD CITY CA 94063-2469

RETURN TO SENDER

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/900,519	07/06/2001	Keith D. Allen	R-615	3963

7590 01/26/2004

DeltaGen, Inc.
1003 Hamilton Avenue
Menlo Park, CA 94025

EXAMINER	
PARAS JR, PETER	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 01/26/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/900,519	ALLEN, KEITH D.	
	Examiner	Art Unit	
	Peter Paras, Jr.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *25-33* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *25-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *20 October 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Applicant's amendment received on 10/20/03 has been entered. Claims 1-24 have been cancelled. New claims 25-33 have been added. Claims 25-33 are pending and are under current consideration.

Specification

The amendment to the brief description of the drawings has been entered.

Sequence Compliance

The instant application is now in sequence compliance.

Upon further consideration the following new grounds of rejection under 35 U.S.C. 101 are necessary:

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 25-33 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility.

The claims are directed to a transgenic mouse whose genome comprises a disruption in the endogenous adrenomedullin receptor gene comprising the nucleotide sequence set forth in SEQ ID NO: 1, wherein the mouse exhibits decreased activity or

increased anxiety as characterized by open field testing. The claims are further directed to a method of producing the same transgenic mouse.

The instant specification has contemplated that the nucleotide sequence set forth in SEQ ID NO: 1 encodes an adrenomedullin receptor. The instant specification has further contemplated that disruption of the nucleotide sequence set forth in SEQ ID NO: 1 in a mouse will produce a phenotype related to an adrenomedullin receptor. The instant specification has purported that such mice may be used to identify agents that modulate or ameliorate a phenotype associated with a disruption in SEQ ID NO: 1.

The instant specification has disclosed a transgenic mouse whose genome comprises a disruption in SEQ ID NO: 1, wherein the mouse exhibits decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field. The claims embrace such a mouse and a method of making the mouse. The instant specification has discussed that phenotypes exhibited by such a transgenic mouse could correlate to a disease or disorder. However, the evidence of record does not provide a correlation between decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field and any disease or disorder. Moreover, while the specification has purported that the nucleotide sequence set forth in SEQ ID NO: 1 encodes an adrenomedullin receptor, the evidence of record has failed to provide a correlation between any adrenomedullin receptor related disease/disorder and decreased activity characterized by reduced

distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field. The specification has provided general assertions that the claimed transgenic mice may be used to identify agents that affect a phenotype related to the mice.

As such, the asserted utility, for the transgenic mouse embraced by the claims, of screening agents that may affect a phenotype of said mouse as provided by the instant specification and encompassed by the claims, does not appear to be specific and substantial. The asserted utility does not appear specific and substantial to the skilled artisan since the evidence of record has not provided any suggestion of a correlation between any adrenomedullin receptor, decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field, and any disease or disorder. Since the evidence of record has not provided a correlation between decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field, the utility of identifying agents that affect decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field is not apparent. The evidence of record has not provided any other utilities for the transgenic mouse embraced by the claims that are specific, substantial, and credible.

The asserted utility of the transgenic mouse embraced by the claims is based on the expectation that disrupting the nucleotide sequence set forth in SEQ ID NO: 1 would result in a detectable phenotype in the mouse. The phenotype observed in the transgenic mice embraced by the claims is decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field. While the phenotypes exhibited by the claimed transgenic mouse are contemplated to be associated with a disease, the association of decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field with any disease has yet to be elucidated. In fact the art suggests that results obtained from behavioral studies, such as the open field test, are greatly influenced by the genetic background of the tested mouse. Crabbe et al (Science, 1999, Vol. 284, pages 1670-1672) observed that laboratory environment and site, test conditions, and genetic strain of a mouse influence the results of behavioral studies. See pages 1670-1671. With regard to the open field test, Crabbe reports that A/J mice were relatively inactive, while C57BL/6 mice were very active. Crabbe further reports that on average mice tested in Edmonton were more active than those tested in Albany or Portland. See page 1671, column 1, the first full paragraph. Crabbe discusses that such inconsistencies in test results can be responsible for observed behavioral phenotypes. Given the inconsistencies in behavioral test results, Crabbe concludes by cautioning that specific behavioral effects observed in mutant (knockout) mice should be not be

uncritically attributed to genetic manipulations prior to repeating testing in different laboratories using different strains of mice, if possible. See page 1672, column 1, paragraphs 2-3. With regard to increased anxiety as related to open field testing, Belzung et al (Behavioural Brain Research, 2001, 125: 141-149) suggest limited usefulness of models of anxiety based on a single gene deletion, which alone can hardly account for a complex condition such as anxiety. See page 147, in the last paragraph. Belzung et al also discuss the differences in anxiety levels among different strains of inbred mice and provide evidence correlating the different genetic backgrounds of the mice and differences in levels of anxiety as measured by the open-field test. See pages 146-147.

Therefore, the reference suggests a need to provide independent evidence of an association of decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field with a disease or disorder. However, neither the specification nor any art of record provides evidence of the existence of a correlation between decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field and a disease or disorder, leaving the skilled artisan to speculate and investigate the uses of the transgenic mouse embraced by the claims. The specification essentially gives an invitation to experiment wherein the artisan is invited to elaborate a functional use for the transgenic mouse embraced by the claims. In light of the above, the skilled artisan would not find the

asserted utility of the transgenic mouse embraced by the claims to be specific and substantial.

Claim Rejections - 35 USC § 112, 1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 25-33 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

In addition to the above, the following grounds of rejection under 35 U.S.C. 112, 1st paragraph are necessary:

Claims 25-33 encompass transgenic non-human animals that comprising a disruption in an endogenous adrenomedullin receptor gene. The claims can be interpreted to read on transgenic mice having either a heterozygous and homozygous disruption in the adrenomedullin receptor gene. While the instant specification has provided guidance correlating a homozygous disruption in the adrenomedullin receptor gene with a phenotype of decreased activity or increased anxiety, the instant specification has not provided guidance correlating a phenotype with a heterozygous

disruption of the adrenomedullin receptor gene. The state of the art at the time of filing was such that one of skill could not predict the phenotype of a knockout mouse (See Moreadith et al., 1997, J. Mol. Med., Vol. 75, pages 208-216). In particular, Moreadith et al. discuss that gene targeting at a particular loci is unpredictable with respect to the resulting phenotype since often the generation of knockout mice, in many instances, changes the prevailing notions regarding the functions of the encoded proteins. Moreadith et al. go on to report that gene targeting at the endothelin loci led to the creation of mice with Hirschsprung's disease instead of the anticipated phenotype (abnormal control of blood pressure). See page 208, column 2, 2nd paragraph. Moens et al. (Development, Vol. 119, pages 485-499, 1993) disclose that two mutations produced by homologous recombination in two different locations of the N-myc gene produce two different phenotypes in mouse embryonic stem cells, one leaky and one null (see abstract). The specification has asserted that the nucleotide sequence set forth in SEQ ID NO: 1 encodes an adrenomedullin receptor. However, given the state of the art it would be difficult to predict any phenotype resulting from disruption of the sequence of SEQ ID NO: 1. The specification discloses that the phenotypes exhibited by transgenic knockout mice comprising a homozygous disruption in the nucleotide sequence set forth in SEQ ID NO: 1 are as follows: decreased activity characterized by reduced distance traveled in an open field or by reduced average velocity in an open field or increased anxiety characterized by reduced average velocity in an open field. See pages 51-52 of the specification. Given the lack of guidance provided by the instant specification, the skilled artisan would know how to use a transgenic knockout

non-human animal that lacks a phenotype, particularly because the instant specification has not provided uses for such; the transgenic mice exhibiting the recited above phenotypes may be used for drug testing according to the instant specification. The specification overcomes the unpredictability in obtaining a phenotype (as discussed above) by correlating a homozygous disruption of the nucleotide sequence set forth in SEQ ID NO: 1 in the genome of a transgenic mouse; however, the claims are not commensurate in scope with the phenotype disclosed in the specification because the claims recite language that reads on transgenic mice that are homozygous or heterozygous for the disruption. As previously discussed only the transgenic mice whose genomes comprise a homozygous disruption of the nucleotide sequence set forth in SEQ ID NO: 1 exhibit the above discussed phenotypes. Given the unpredictable nature of a phenotype that results from disruption of a nucleotide sequence and the lack of guidance provided by the instant specification for use of a transgenic mouse lacking a phenotype, it would have required undue experimentation for the skilled artisan to make and use the invention as claimed.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Peter Paras, Jr., whose telephone number is (571) 272-0732. The examiner can normally be reached Monday-Friday from 8:30 to 4:30 (Eastern time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached at 571-272-0804. Papers related to this application may be submitted by facsimile transmission. Papers should be faxed via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Official Fax Center number is (703) 872-9306.

Inquiries of a general nature or relating to the status of the application should be directed to Dianiece Jacobs whose telephone number is (571) 272-0532.

Peter Paras, Jr.

Art Unit 1632

PETER PARAS
PATENT EXAMINER

Notice of References Cited	Application/Control No. 09/900,519	Applicant(s)/Patent Under Reexamination ALLEN, KEITH D.	
	Examiner Peter Paras, Jr.	Art Unit 1632	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Crabbe et al, 1999, Science, 284: 1670-1672
	V	Belzung et al, 2001, Behavioural Brain Research, 125: 141-149.
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

15. D. G. Hangauer, A. F. Monzingo, B. W. Matthews, *Biochemistry* **23**, 5730 (1984).
16. O. Dideberg *et al.*, *Nature* **299**, 469 (1982); J. M. Ghuysen, J. Lamotte-Brasseur, B. Joris, G. D. Shockman, *FEBS Lett.* **342**, 23 (1994).
17. W. Stocker and W. Bode, *Curr. Opin. Struct. Biol.* **5**, 383 (1995).
18. K. L. Constantine *et al.*, *J. Mol. Biol.* **223**, 281 (1992); A. R. Pickford, J. R. Potts, J. R. Bright, I. Phan, I. D. Campbell, *Structure* **5**, 359 (1997).
19. I. E. Collier, P. A. Krasnov, A. Y. Strongin, H. Birkedal-Hansen, G. I. Goldberg, *J. Biol. Chem.* **267**, 6776 (1992).
20. L. Bányai, H. Tordai, L. Patthy, *ibid.* **271**, 12003 (1996).
21. F. Willenbrock *et al.*, *Biochemistry* **32**, 4330 (1993); M. W. Olson, D. C. Gervasi, S. Mobashery, R. Fridman, *J. Biol. Chem.* **272**, 29975 (1997); C. M. Overall *et al.*, *ibid.* **274**, 4421 (1999).
22. J. Hodgson, *Biotechnology* **13**, 554 (1995); A. E. Yu, R. E. Hewitt, E. W. Connor, W. G. Stetler-Stevenson, *Drugs Aging* **11**, 229 (1997); S. A. Watson and G. Tierney, *Biodrugs* **9**, 325 (1998).
23. XDS [W. Kabsch, *J. Appl. Crystallogr.* **21**, 916 (1988)]; CCP4 programs [CCP4, Collaborative Computational Project No. 4, Daresbury, UK, *Acta Crystallogr. D* **50**, 760 (1994)]; SFCHECK [A. A. Vagin, J. Richelle, S. J. Wodak, *ibid.* **55**, 191 (1999)]; AMORE [J. Navaza, *Acta Crystallogr. A* **50**, 157 (1994)]; O [T. A. Jones, J.-Y. Zou, S. W. Cowan, M. Kjeldgaard, *ibid.* **47**, 110 (1991)]; and X-PLOR [A. T. Brünger, *X-PLOR, Version 3.1: A System for X-Ray Crystallography and NMR* (Yale Univ. Press, New Haven, CT, 1992)].
24. Figures 1, 2A, 3, A and B, and 4A were made with MOLSCRIPT [P. J. Kraulis, *J. Appl. Crystallogr.* **24**, 946 (1991)] and RASTER3D [E. A. Merrit and M. E. P. Murphy, *Acta Crystallogr. D* **50**, 869 (1994)]. Figures 3, C and D, and 4B were made with GRASP [A. Nichols, K. A. Sharp, B. Honig, *Proteins* **11**, 281 (1991)].
25. Supported by grants from the Swedish Cancer Foundation, EC project BMH4-CT 96-0012, Novo Nordisk Foundation, and Hedlund's Foundation. We thank Tiina Berg, Ilkka Miinalainen, and Kristian Tryggvason for technical assistance with insect cell cultures. We are also grateful to Richard Kahn and Tatjana Sandalova for assistance with the data collection. Beam time was provided by the ESRF.

21 December 1998; accepted 28 April 1999

Genetics of Mouse Behavior: Interactions with Laboratory Environment

John C. Crabbe,[1]* Douglas Wahlsten,[2] Bruce C. Dudek[3]

Strains of mice that show characteristic patterns of behavior are critical for research in neurobehavioral genetics. Possible confounding influences of the laboratory environment were studied in several inbred strains and one null mutant by simultaneous testing in three laboratories on a battery of six behaviors. Apparatus, test protocols, and many environmental variables were rigorously equated. Strains differed markedly in all behaviors, and despite standardization, there were systematic differences in behavior across labs. For some tests, the magnitude of genetic differences depended upon the specific testing lab. Thus, experiments characterizing mutants may yield results that are idiosyncratic to a particular laboratory.

Targeted and chemically induced mutations in mice are valuable tools in biomedical research, especially in the neurosciences and psychopharmacology. Phenotypic effects of a knockout often depend on the genetic background of the mouse strain carrying the mutation (*1*), but the effects of environmental background are not generally known.

Different laboratories commonly employ their own idiosyncratic versions of behavioral test apparatus and protocols, and any laboratory environment also has many unique features. These variations have sometimes led to discrepancies in the outcomes reported by different labs testing the same genotypes for ostensibly the same behaviors (*2*). Previous studies could not distinguish between interactions arising from variations in the test situation itself and those arising from subtle environmental differences among labs. Usually, such differences are eventually resolved by repetition of tests in multiple labs. However, null mutants and transgenic mice are often scarce and tend to be behaviorally characterized in a single laboratory with a limited array of available tests.

We addressed this problem by testing six mouse behaviors simultaneously in three laboratories (Albany, New York; Edmonton, Alberta, Canada; and Portland, Oregon) using exactly the same inbred strains and one null mutant strain (*3*). We went to extraordinary lengths to equate test apparatus, testing protocols, and all possible features of animal husbandry (*4*). One potentially important feature was varied systematically. Because many believe that mice tested after shipping from a supplier behave differently from those reared in-house, we compared mice either shipped or bred locally at the same age (77 days) starting at the same time (0830 to 0900 hours local time on 20 April 1998) in all three labs (*5*). Each mouse was given the same order of tests [Day 1: locomotor activity in an open field; Day 2: an anxiety test, exploration of two enclosed and two open arms of an elevated plus maze; Day 3: walking and balancing on a rotating rod; Day 4: learning to swim to a visible platform; Day 5: locomotor activation after cocaine injection; Days 6 to 11: preference for drinking ethanol versus tap water (*6*)].

Despite our efforts to equate laboratory environments, significant and, in some cases, large effects of site were found for nearly all variables (Table 1). Furthermore, the pattern of strain differences varied substantially among the sites for several tests. Sex differ-

Table 1. Statistical significance and effect sizes for selected variables in the multisite trial. Color of cell depicts Type I error probability or significance of main effects and two-way interactions from 8 × 2 × 3 × 2 analyses of variance: blue, $P < 0.00001$; purple, $P < 0.001$; gold, $P < 0.01$; dashes with no shading, $P > 0.01$. Cell entries are effect sizes, expressed as partial omega squared, the proportion of variance accounted for by the factor or interaction if only that factor were in the experimental design (range = 0 to 1.0). Multiple R^2 (unbiased estimate) gives the proportion of the variance accounted for by all factors. For the water escape task, results are based on only seven strains because most A/J mice never escaped because of wall-hugging. We recognize that the issue of appropriate alpha level correction for multiple comparisons is contentious. Details of the statistical analyses are available on the Web site (*4*), including a discussion of our rationale for presenting uncorrected values in this table.

Task	Measure	Eight Genotypes	Three Sites	Two Sexes	Local vs Shipped	Genotype x Site	Genotype x Sex	Genotype x Ship	Multiple R^2
Open field	Distance in 15 min	.600	.157	----	----	.059	.045	----	.604
Open field	# vertical movements	.788	.281	.039	----	----	----	----	.772
Cocaine	Difference from Day 1	.338	.053	----	----	.068	----	----	.342
Plus maze	Total arm entries	.385	.327	----	----	.210	----	----	.660
Plus maze	Time in open arms	.082	.212	----	----	.066	----	----	.266
Water maze	Mean escape latency	.221	----	----	.026	----	----	----	.177
Alcohol preference	Alcohol consumed (g kg)	.483	----	.043	----	----	----	----	.451
Bod. size	Weight (g)	.408	.204	.637	----	.071	.070	----	.698

[1]Portland Alcohol Research Center, Department of Veterans Affairs Medical Center and Department of Behavioral Neuroscience, Oregon Health Sciences University, Portland, OR 97201, USA. [2]Department of Psychology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9. [3]Department of Psychology, State University of New York at Albany, Albany, NY 12222, USA.

*To whom correspondence should be addressed. E-mail: crabbe@ohsu.edu

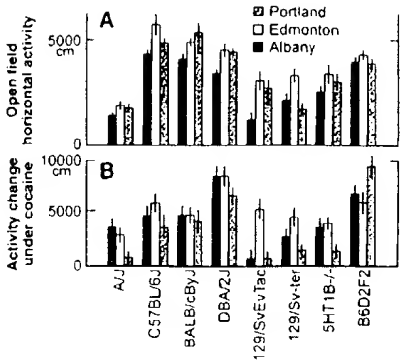


Fig. 1. Group means (±SEM for n = 16 mice) for activity in a 40 cm by 40 cm open field for eight strains tested at the same time of day in identical apparatus in three laboratories. (**A**) Horizontal distance (centimeters) traveled in 15 min on the first test on Day 1. (**B**) Cocaine-induced activation, expressed as the difference between horizontal activity (centimeters in 15 min) after cocaine (20 mg/kg) on Day 5 minus the score on Day 1.

ences were only occasionally detected, and, much to our surprise, there were almost no effects of shipping animals before testing. Large genetic effects on all behaviors were confirmed, which is not surprising because we chose strains known to differ markedly on these tasks.

Results for locomotor activity and the effect of a subsequent cocaine injection on locomotion are shown in Fig. 1. Expected strain differences in undrugged activity were found: A/J mice were relatively inactive at all three sites, whereas C57BL/6J mice were very active. An effect of laboratory was also found: mice tested in Edmonton were, on average, more active than those tested in Albany or Portland. In addition, the pattern of genetic differences depended on site. For example, 129/SvEvTac mice tested in Albany were very inactive compared to their counterparts in other labs. Similar results were seen for sensitivity to cocaine stimulation. For example, B6D2F2 mice were very responsive (and A/J mice quite insensitive) to cocaine in Portland, but not at other sites.

In the elevated plus maze, a very similar pattern was seen: strong effects of genotype, site, and their interaction. This was true both for activity measures and for time spent in open arms, the putative index of anxiety (Fig. 2). For total arm entries, the testing laboratory was particularly important for the 5-HT_{1B} knockout mice versus their wild-type 129/Sv-ter background controls. Knockout mice had greater activity than wild types in Portland and tended to have less activity in Albany, while not differing in Edmonton. Edmonton mice of all strains spent more time in open arms (lower anxiety). Portland mice also spent less time in open arms, but this was especially true for strains A/J, BALB/cByJ, and the B6D2F2 mice.

Although the testing laboratory was an important variable, there was a good deal of consistency to the genetic results as well. For example, comparison of the genotype means (averaged over sites) for the initial 5 min of the activity test on Day 1 with the total arm entry scores from the plus maze yielded a high correlation between strains (r = 0.91, $P < 0.002$). This indicates that a strain's characteristic activity in novel apparatus is robust and occurs in different apparatus as well as different labs (*7*).

For some behaviors, laboratory environment was not critical. For example, ethanol drinking scores were closely comparable across all three labs, and genotypes alone accounted for 48% of the variance (Table 1 and Fig. 3). The genetic differences showed the well-known pattern of C57BL/6J mice strongly preferring and DBA/2J mice avoiding ethanol (*8*). Females drank more, as is also well known (*8*), but there were no significant effects of site, shipping, or any other interactions. Unlike the other five tests, ethanol preference testing extended over 6 days in the home cage and involved a bare minimum of handling mice by the experimenter.

For some measures, the difference between 5-HT_{1B} null mutant and wild-type mice depended on the specific laboratory environment. In Edmonton, for example, no difference was observed between +/+ and −/− mice in distance traveled in the activity monitor, whereas there was greater activity in the knockouts at the other two sites, especially Portland (P = 0.002). In the elevated plus maze, knockouts were considerably more active than wild types only in Portland (Fig. 2A; P = 0.02).

The numbers of mice we tested made formal statistical assessment of reliability infeasible, but it would be important to know whether each laboratory would obtain essentially the same strain-specific results if this experiment were repeated. Because our experiment included an internal replication, we estimated the lower bounds of reliability for each site separately by correlating the mean scores for each strain (collapsed over sex and shipping group) obtained during the two replicates of the experiment. These correlations differed depending upon the behavior, and were consonant with the relative importance of genotype in the overall analysis. For example, for locomotor activity, the correlations were 0.97, 0.74, and 0.87 for the three sites. For open-arm time on the plus maze, possibly the most intrinsically unstable task we employed, the correlations were lower (0.32, 0.52, and 0.26). These can be compared to correlations for body weight, which can serve as a type of control variable not influenced by idiosyncratic dynamics of the test situation (0.83, 0.74, and 0.90). No site had generally higher or lower reliability than the others, and formal analyses of replication in analyses of variance indicated no strong interactions of strain by replication. We conclude that reasonable estimates of strain-specific scores are highly dependent on behavioral endpoint, and that some behaviors are highly stable.

Several sources of these laboratory-specific behavioral differences could be ruled out by the rigor of the experimental design. For example, Edmonton mice might have been

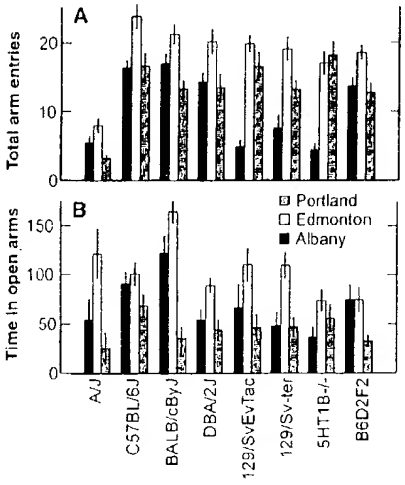


Fig. 2. Group means (±SEM for n = 16 mice) for behavior videotaped for 5 min on elevated plus mazes having two open and two enclosed arms. (**A**) Total number of entries into any arm (defined as all four limbs in the arm). (**B**) Time (seconds) spent in the two open arms during the 300-s test. Smaller amounts of time indicate higher levels of anxiety.

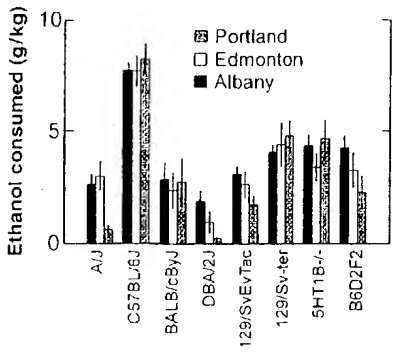


Fig. 3. Mean (±SEM) ethanol consumed per day, expressed as grams per kilogram body weight, over 4 days of an ethanol preference test where each mouse had free access to two drinking bottles, one with local tap water and the other with 6% ethanol in tap water.

more sensitive to cocaine-induced locomotion because the source of cocaine differed from the other two sites (*4*), but this could not explain the relatively marked response of the three 129-derived strains in Edmonton only. However, specific experimenters performing the testing were unique to each laboratory and could have influenced behavior of the mice. The experimenter in Edmonton, for example, was highly allergic to mice and performed all tests while wearing a respirator—a laboratory-specific (and uncontrolled) variable.

Whether animals were bred in each laboratory or shipped as adults 5 weeks before testing had no consistent influence on results in this experiment. Shipped animals took routes of varying duration and difficulty. For example, some Taconic mice were trucked to Albany from nearby Germantown, New York, whereas others spent 2 days in transit during a flight in midwinter to Edmonton. At least in this experiment, allowing animals a lengthy period of acclimation to new quarters was sufficient to overcome any strong effects of putative shipping stress on subsequent behavior.

These results support both optimistic and pessimistic interpretations. Seen optimistically, genotype was highly significant for all behaviors studied, accounting for 30 to 80% of the total variability, and several historically documented strain differences were also seen here. In general, we conclude that very large strain differences are robust and are unlikely to be influenced in a major way by site-specific interactions. However, a more cautious reading suggests that for behaviors with smaller genetic effects (such as those likely to characterize most effects of a gene knockout), there can be important influences of environmental conditions specific to individual laboratories, and specific behavioral effects should not be uncritically attributed to genetic manipulations such as targeted gene deletions.

When studying mutant mice, relatively small genetic effects should first be replicated locally before drawing conclusions (*9*). We further recommend that, if possible, genotypes should be tested in multiple labs and evaluated with multiple tests of a single behavioral domain (such as several tests of anxiety-related behavior) before concluding that a specific gene influences a specific behavioral domain. We also suggest the possibility that laboratory-specific effects on genetic differences will affect phenotypes other than behaviors to an extent similar to that we report.

It is not clear whether standardization of behavioral assays would markedly improve future replicability of results across laboratories. Standardization will be difficult to achieve because most behaviorists seem to have differing opinions about the "best" way to assay a behavioral domain. For example, two of us typically test behavior during the light phase of the animals' cycle, whereas the third typically tests during the dark phase (but switched to the light phase for this study). Which apparatus specifications or test protocol to employ is also a subject of differing opinion. There is a risk of prematurely limiting the "recommended" tests in a domain to those deemed "industry standard," because this may constrain the intrinsic richness of a domain and obscure interesting interactions. On the other hand, increased communication and collaboration between the molecular biologists creating mutations and behavioral scientists interested in the psychological aspects of behavioral testing will benefit both groups.

References and Notes

1. M. Sibilia and E. F. Wagner, *Science* **269**, 234 (1995); R. Gerlai, *Trends Neurosci.* **19**, 177 (1996); M. Nguyen *et al.*, *Nature* **390**, 78 (1997).
2. It has been known for some time that comparisons of multiple genotypes on learning-related tests do not always yield consistent results across laboratories [D. Wahlsten, in *Psychopharmacology of Aversively Motivated Behavior*, H. Anisman and G. Bignami, Eds. (Plenum, New York, 1978), pp. 63–118]. For another example, the Crabbe laboratory has reported that C57BL/6 mice show a small enhancement of locomotor activity after low doses of ethanol, while the Dudek laboratory finds no such stimulant response [J. C. Crabbe *et al.*, *J. Comp. Physiol. Psychol.* **96**, 440 (1982); B. C. Dudek and T. J. Phillips, *Psychopharmacology* **101**, 93 (1990)]. Similar variation has been reported in other measures of activity in various laboratories and apparatus [J. M. LaSalle and D. Wahlsten, in *Techniques for the Genetic Analysis of Brain and Behavior: Focus on the Mouse*, D. Goldowitz, D. Wahlsten, R. E. Wimer, Eds. (Elsevier, Amsterdam, 1992), pp. 391–406].
3. We tested males and females from the inbred strains: A/J, BALB/cByJ, C57BL/6J, DBA/2J, 129/Sv-ter, and 129/SvEvTac; the F_2 hybrid cross of C57BL/6J and DBA/2J (B6D2F2); and the serotonin receptor subtype null mutant, 5-$HT_{1B}^{-/-}$, which is maintained on the 129/Sv-ter background. Mice were obtained from the Jackson Laboratory (Bar Harbor, ME), Taconic Farms (Germantown, NY), or the colonies of R. Hen (Columbia University, New York, NY). Because many targeted deletions are placed on the 129/SvEvTac background, we included this close relative of 129/Sv-ter. The genealogy of many 129 substrains has recently been discussed [E. M. Simpson *et al.*, *Nature Genet.* **16**, 19 (1997); D. W. Threadgill, D. Yee, A. Matin, J. H. Nadeau, T. Magnuson, *Mamm. Genome* **8**, 390 (1997)].
4. Details of procedures and test protocols are given in the Web site for this study (www.albany.edu/psy/obssr). Variables explicitly equated across laboratories included apparatus, exact testing protocols, age of shipped and laboratory-reared mice, method and time of marking before testing, food (Purina 5001; Purina 5000 for breeders), bedding (Bed-o-cob, 1/4 inch; Animal Specialties, Inc., Hubbard, OR), stainless steel cage tops, four to five mice per cage, light/dark cycle, cage changing frequency and specific days, male left in cage after births, culling only of obvious runts, postpartum pregnancy allowed, weaned at 21 days, specific days of body weight recording, and gloved handling without use of forceps. Unmatched variables included local tap water, requirement of filters over cage tops in Portland only, variation of physical arrangement of colonies and testing rooms across sites, different air handling and humidity, and different sources of batches of cocaine and alcohol.
5. All breeding stock was shipped on 2 or 3 December 1997, and mating pairs were set simultaneously on 13 January 1998 in all labs to provide "unshipped" mice for testing. On 15 to 17 March 1998, a second batch of mice from each genotype was shipped to each laboratory. These "shipped" mice, age matched with the unshipped cohort already in place, were allowed to acclimate to the laboratory for 5 weeks before testing commenced. We tested 128 mice in each lab, in two groups of 64 separated by 1 week. With an $n = 4$ mice in each genotype/shipping condition/sex/laboratory condition, we had 16 mice per group for the crucial genotype × laboratory comparisons. This sample size gave us statistical power of 90% to detect modest interactions of genotype × laboratory when Type I error probability was set at 0.01 [J. Cohen, *Statistical Power Analysis* (Erlbaum, Hillsdale, NJ, 1988); D. Wahlsten, *Behav. Brain. Sci.* **13**, 109 (1990)]. For results of analysis of variance, we report only effects significant at $P < 0.01$. The Web site in (*4*) provides detailed protocols used for each test, descriptions of the laboratory conditions rigorously equated across labs, and raw data that may be examined for other interesting patterns.
6. AccuScan Digiscan monitors (AccuScan Instruments, Columbus, OH) were generously loaned to D. Wahlsten by R. H. Kant to match those available in the other two laboratories. AccuScan also provided all sites with rotarod apparatus. Mouse-scaled water mazes and elevated plus mazes were constructed by D. Wahlsten and shipped to the other two labs. On the first test day, each mouse was tested for 15 min in a Digiscan open-field monitor in a dark, sound-attenuated chamber. On Day 2, each mouse was videotaped for 5 min in an elevated plus maze. On Day 3, mice were given 10 trials on a rotarod set to accelerate from 0 to 100 rpm in 75 s. After all mice had been tested on the rotarod, mice were pretrained briefly to escape from the water maze. On Day 4, mice were given eight massed trials of escape learning to a visible platform in the water maze. On Day 5, the activity test was repeated immediately following an ip injection of 20 mg of cocaine per kilogram. After 2 days of rest, mice were individually housed, given only tap water for 2 days, and then tested for 4 days for drinking of 6% ethanol in tap water versus tap water alone.
7. J. Flint *et al.*, *Science* **269**, 1432 (1995); S. R. Mitchell, J. K. Belknap, J. C. Crabbe, unpublished observations.
8. G. E. McClearn and D. A. Rodgers, *Q. J. Stud. Alcohol* **20**, 691 (1959); J. K. Belknap, J. C. Crabbe, E. R. Young, *Psychopharmacology* **112**, 503 (1993); L. A. Rodriguez *et al.*, *Alcohol. Clin. Exp. Res.* **19**, 367 (1995).
9. It was previously reported that 5-HT_{1B} null mutant mice drank much more alcohol than the 129/Sv-ter wild-type strain [J. C. Crabbe *et al.*, *Nature Genet.* **14**, 98 (1996)]. In the experiments here, no site detected this difference (Fig. 3 and Table 1). The original outcome was replicated four times (J. C. Crabbe *et al.*, unpublished data). It is possible that residual polymorphisms for genes segregating in the 129/SvPas substrain that served as the original source of the embryonic stem cell line and in the 129/Sv-ter substrain to which the null mutant was crossed have subsequently been fixed differentially in the 5-HT_{1B} +/+ and −/− strains maintained at Columbia University (*3*). If so, these genes must exert very large epistatic effects on the 1B gene deletion's phenotypic effects on drinking (*1*). Alternatively, some undetected variable (for example, a change in animal care personnel) may have occurred specifically at the Portland site between the original (1995–96) observations and the current experiments.

10. Supported by the Office of Behavioral and Social Sciences Research, NIH, as supplements to grants AA10760 (J.C.C.) and DA10731 (J. Marley and B.C.D., co-principal investigators), and by the Natural Sciences and Engineering Research Council of Canada Grant # 45825 (D.W.), the Department of Veterans Affairs (J.C.C.), and a K02 Award to B.C.D. AA00170. We thank R. H. Kant at AccuScan for the generous loan of equipment and R. Hen for providing the serotonin receptor mutants. We appreciate the comments of C. Cunningham, R. A. Harris, J. Janowsky, and G. Westbrook on a draft of this manuscript. We also thank S. Boehm II, S. Burkhart-Kasch, J. Dorow, S. Doerksen, C. Downing, J. Fogarty, K. Henricks, C. McKinnon, C. Merrill, P. Metten, C. Nolte, T. Phillips, M. Schalomon, J. Schlumbohm, J. Sibert, J. Singh, and C. Wenger for valuable assistance.

1 February 1999; accepted 7 May 1999

ELSEVIER

Behavioural Brain Research 125 (2001) 141–149

BEHAVIOURAL BRAIN RESEARCH

www.elsevier.com/locate/bbr

Measuring normal and pathological anxiety-like behaviour in mice: a review

Catherine Belzung [a,*], Guy Griebel [b]

[a] *EA 3248 Psychobiologie des émotions, UFR Sciences et Techniques, Parc Grandmont, Avenue Monge, F-37200 Tours, France*
[b] *CNS Research Department, Sanofi-Synthelabo, Bagneux, France*

Received 13 October 2000; accepted 8 February 2001

Abstract

Measuring anxiety-like behaviour in mice has been mostly undertaken using a few classical animal models of anxiety such as the elevated plus-maze, the light/dark choice or the open-field tests. All these procedures are based upon the exposure of subjects to unfamiliar aversive places. Anxiety can also be elicited by a range of threats such as predator exposure. Furthermore, the concepts of 'state' and 'trait' anxiety have been proposed to differentiate anxiety that the subject experiences at a particular moment of time and that is increased by the presence of an anxiogenic stimulus, and anxiety that does not vary from moment to moment and is considered to be an 'enduring feature of an individual'. Thus, when assessing the behaviour of mice, it is necessary to increase the range of behavioural paradigms used, including animal models of 'state' and 'trait' anxiety. In the last few years, many mice with targeted mutations have been generated. Among them some have been proposed as animal models of pathological anxiety, since they display high level of anxiety-related behaviours in classical tests. However, it is important to emphasise that such mice are animal models of a single gene dysfunction, rather than models of anxiety, per se. Inbred strains of mice, such as the BALB/c line, which exhibits spontaneously elevated anxiety appear to be a more suitable model of pathological anxiety.

Keywords: Anxiety; Mouse; Openfield; Elevated plus maze; Predator-elicited defensive responses; Free exploration test; BALB/c mice

1. Introduction

The discovery of benzodiazepines (BZs) in the early sixties and their considerable commercial success in the treatment of anxiety has fueled the development of numerous animal models of anxiety. Unfortunately, because BZs were the only anxiolytic agents marketed at that time, the predictive validity of these initial models has been mainly based on their ability to detect the pharmacological action of BZs. This became evident in the early eighties, when non-BZ anxiolytics, such as the 5-HT_{1A} receptor partial agonist buspirone, were found inactive in some anxiety tests, in particular conflict procedures. At that time, unconditioned conflict tests such as the elevated plus-maze were developed. Later, a second difficulty appeared, when it became evident that anxiety is not a unitary phenomenon but could be divided in various forms including 'state' and 'trait' anxiety, 'normal' and 'pathological' anxiety. These various forms have been shown to be differentially sensitive to pharmacological challenge. Therefore, when measuring anxiety in animals, it would be useful to have information on the type of anxiety processes which may be involved in a given test. These models are now extensively used not only to predict the clinical efficacy of pharmacological treatments, but also to phenotype the behaviour of transgenic or knockout mice.

The aim of the present paper is to consider animal models of both 'normal' and 'pathological' anxiety. Therefore, we will first give a tentative definition of anxiety, and review the validity criteria of animal models, before presenting animal models of 'normal' and 'pathological' anxiety. Only animal models using mice as subjects will be considered.

* Corresponding author. Tel.: +33-247-36-6994; fax: +33-247-36-7285.
E-mail address: belzung@univ-tours.fr (C. Belzung).

0166-4328/01/$ - see front matter
PII: S0166-4328(01)00291-1

2. Tentative definition of anxiety

Fear and anxiety are here, respectively, defined as the response of a subject to real or potential threats that may impair its homeostasis. This response may include physiological (increase in heart rate, blood pressure etc.), as well as behavioural (inhibition of ongoing behaviours, scanning, avoidance of the source of danger, etc.) parameters. When this response is excessive or maladaptive, it involves 'pathological' anxiety. On a clinical level, the DSM IV [25] describes various forms of anxiety disorders, including phobias, generalised anxiety, post-traumatic stress, panic and obsessive-compulsive disorders.

3. Validity criteria of an animal model

What is an animal model of a human behavior? According to McKinney, animal models are 'experimental preparations developed in one species for the purpose of studying phenomena occurring in another species' [57]. Kaplan [45] added that a model may be valid if it has the same structure as the human behavior or pathology, that is whenever a relation holds between two elements of the animal model, a corresponding relation may hold between the corresponding elements of the human behavior.

Other authors [58,84,90] have proposed additional criteria. According to them, an animal model should fit predictive validity (pharmacological correlation), face validity (isomorphism) and construct validity (homology and similarity of underlying neurobiological mechanisms) to be suitable for research.

3.1. Predictive validity

Predictive validity implies that the animal model should be sensitive to clinically effective pharmacological agents. Conversely, anxiogenic compounds should elicit opposite effects, while agents that have no effect in the clinic should have no effect in these tests.

It is important to note that this involves that a given model may include both variables that are increased by anxiety as well as variables that are decreased by anxiety. For example, when an animal is confronted with a potent source of danger, it displays increased risk assessment behaviours and decreased exploratory activity. In many cases, only the second category of variables are recorded so that an increase in anxiety can be confounded with a non specific inhibition of activity, such as sedation, ataxia, myorelaxation, pre-ictal prostration or even toxic effects induced by the treatment. Many anxiolytics produce such non specific effects: This is particularly striking with BZs which display marked sedative effects at high doses.

Even if the 'predictive validity' criterion seems satisfactory, its relevance can be questioned. Species differences in pharmacokinetic or pharmacodynamic can be observed. For example, in man an important age-related increase of distribution of diazepam has been described, while this does not occur in the same proportions in rats [85]. Furthermore, tachykinin NK_1 receptor antagonists have been proposed for the treatment of anxiety. However, species differences have been described in the NK_1 receptor pharmacology. For example, the NK_1 receptor antagonist, CP-96 345 has high affinity for the human receptor, but shows low affinity for the rat NK_1 receptor. Furthermore, in the human brain, NK_1 receptors are widely expressed throughout areas involved in the modulation of emotional processes, and there is evidence suggesting that SP is co-expressed with 5-HT, a neurotransmitter involved in anxiety-related processes, whereas co-expression seems to be absent in the rat brain (see [78]). Therefore, NK_1 receptor ligands may have different anxiety-modulating properties depending on the species.

3.2. Face validity

This criterion implies that the anxiety response observed in the animal model should be identical to the behavioural and physiological responses observed in human. This indicates that the expression of a given emotion is supposed to be similar across species. The physiological expression of anxiety (e.g. increase in heart rate, blood pressure, hyperthermia) is a good example. However, as for the behavioural responses, the patterns much vary across species. The behavioural repertoire of mice is of course very different from the human ethogram, which includes the verbal aspect that is absent in rodents. For example, when confronted with a threat, subjects may tend to escape: the consequence of this behaviour is to avoid the source of danger and consequently to preserve their homeostasis. The behavioural responses used to escape may vary across species: fishes may swim, birds may fly, and human may run. In fact, the possibility of an isomorphism between human and animal behaviours has to be placed in the context of the theory of evolution, suggesting that a given pattern may be selected according to its survival value. It is rather important to note here that natural selection operates on the consequences of the behaviour, rather then on the behaviour per se [81], so that the consequences of the behavioural pattern, rather than the behavioural item per se, may be isomorphic.

3.3. Construct validity

This criterion relates to the similarity between the theoretical rationale underlying the animal model and

the human behaviour. This requires that the etiology of the anxiety behaviour and the biological factors underlying anxiety may be similar in animals and humans.

This criteria seems easy to fulfill for the psychological factors underlying 'normal' anxiety, as in both species this behaviour is induced by a threatening stimulus. It is evident that the nature of the danger may vary across species but the important point is that it causes a threat for the subject's homeostasis. This is not only true for proximal factors of causation but also for distal factors that can contribute to increase subject's sensibility toward threats. For example, impoverishment of the breeding conditions, deficient maternal care during perinatal period or early maternal separation, may induce an increase in anxiety in the individuals when adult, both in animals and in humans ([15,17,86]). However, this criterion seems rather difficult to fulfill for pathological anxiety as in most cases (except for post-traumatic stress disorder, a disorder defined by its etiology), the psychological factors underlying the disorder have not been described.

4. 'Normal' anxiety and 'pathological' anxiety

Two rather opposite conceptions have been proposed as to the relationship between the 'normal' and 'pathological' state of a subject. In fact, pathology can be described either as a quantitative variation of a normal state, or as a qualitative variation. According to the first conception, 'pathological' anxiety might be considered as an excess of 'normal' anxiety. The second conception of the relationship between normality and pathology proposes that there is a qualitative, rather than a quantitative variation when passing from the one state to the other. This last conception corroborates that proposed by Canguilhem [16]. In fact, this seems to be the case in anxiety disorders as 'pathological' anxiety rarely includes excess of 'normal' anxiety and as anxiety disorders are not released by the same treatment than 'normal' anxiety.

5. Mouse models of anxiety

Over the past three decades, a bewildering diversity of tests has been developed which claim face, construct and/or predictive validity as animal models of anxiety disorders (for review, see [73,89]). While most of these procedures use rats as subjects, a few of them have been validated with mice. Most of them involve exposure of subjects to external (e.g. cues earlier paired with footshock, bright light, predator) or internal (e.g. drug states) stimuli that are assumed to be capable of inducing anxiety in animals. Since none of these models involves pathological anxiety-related behaviors, Lister has described them as animal models of 'state' anxiety [54]. In such procedures, subjects experience anxiety at a particular moment in time and it is increased by the presence of anxiogenic stimulus. The last few years have seen the emergence of models of 'pathological' anxiety, which are often referred to as 'trait' anxiety tests. Unlike 'state' anxiety, 'trait' anxiety does not vary from moment to moment and is considered to be an enduring feature of an individual. As will be shown below, these models either use rodents that were selected for emotional reactivity or employ receptor knockout mice which exhibit phenotypic changes indicative of increased anxiety.

5.1. Mouse models of 'normal' or 'state' anxiety

There are several excellent review articles that have described and discussed extensively these models (see, for example, [54,73,79,84]). Table 1 gives an overview of the existing mouse models of 'state' anxiety. While the vast majority employ behavioral methods, the type of behavior studied varies considerably. They can be grouped into two main subclasses: the first involves animals' conditioned responses to stressful and often painful events (e.g. exposure to electric footshock); the second includes ethologically based paradigms and involves animals' spontaneous or natural reactions (e.g. flight, avoidance, freezing) to stress stimuli that do not explicitly involve pain or discomfort (e.g. exposure to a

Table 1
Mouse models of 'normal' or 'state' anxiety

Conditioned response tests	Unconditioned response tests
(1) Conflict tests	(1) Exploration tests
(a) Operant punishment paradigm	(a) Elevated plus-maze
(b) Punished drinking	(b) Holeboard
	(c) Light/dark choice task
	(d) Mirrored chamber
	(e) Open field
	(f) Staircase test
	(g) Zero-maze
(2) Others	(2) Social tests
(a) Active/passive avoidance	(a) Agonistic behavior
(b) Conditioned ultrasonic vocalization	(b) Separation-induced ultrasonic vocalizations
(c) Defensive burying	(c) Social interaction
	(3) Others
	(a) Acoustic startle response
	(b) Hot plate
	(c) Mouse defense test battery
	(d) Shock-induced ultrasonic vocalizations
	(e) Stress-induced changes in physiological parameters

Adapted and updated from [36,73].

novel highly illuminated test chamber or to a predator). The majority of studies using mouse models of 'normal' anxiety employ unconditioned-based procedures. Among these, the elevated plus-maze has become one of the most popular behavioral test for research on anxiety [55,74]. As pointed out by Rodgers [73], this popularity is mainly due to practical rather than theoretical reasons, because the elevated plus-maze permits a quick screening of anxiety-modulating drugs or mouse genotypes without training or involvement of complex schedules. Briefly, in this situation, mice generally taken straight from their home cages, will show a pattern of behavior characterized by open-arm avoidance. This tendency is suppressed by anxiolytics and potentiated by anxiogenic agents. Unfortunately, the plus-maze behavior patterns may be influenced by variations in test parameters (e.g. species, housing conditions, time of testing, lighting level, method of scoring) that do not necessarily become clear, even with close scrutiny of published reports [41]. As a result, the vast literature on the elevated plus-maze yielded inconsistent findings. Serotonin (5-HT)-modulating compounds are particularly prone to discrepancies in the plus-maze. For example, a number of research groups have found that selective 5-HT_{1A} receptor agonists (e.g. 8-OH-DPAT, buspirone) display anxiolytic-like effects in this test [6,11,53,63,75], whereas others have reported a lack of activity [80] or even an anxiogenic-like profile [59]. In this context, Rodgers and Johnson [76] have developed and refined an 'ethological' version of the mouse plus-maze that incorporates specific behavioral postures (e.g. risk assessment, head-dipping) together with conventional spatiotemporal measures of open-arm avoidance. Studies using this modified version of the plus-maze showed that risk assessment measures are generally more sensitive to drug effects than are avoidance measures [73]. For example, comparison between BZ and 5-HT_{1A} anxiolytics revealed differences in anxiolytic-like profiles that may not be detected by conventional scoring. Indeed, while both classes of drugs share the ability to reduce risk assessment, only BZs decrease selectively (i.e. at non motor-impairing doses) open-arm avoidance. Hopefully, the inclusion of ethological-based parameters in plus-maze studies may yield more consistent findings than those using the standard version of this test.

Besides the elevated plus-maze, there is a another murine model of 'state' anxiety that uses extensive ethological analysis to generate more comprehensive behavioral profiles following drug treatment, namely the mouse defense test battery (MDTB) [14,35]. The suggestion has been made many times that defensive behaviors of lower mammals constitute a significant model for understanding human emotional disorders [12]. Defensive behaviors occur in response to a number of threatening stimuli, including predators, attacking conspecifics, and dangerous objects or situations. Such behaviors can readily be studied in wild rats, wild mice or in SWISS mice which show a complete defensive repertoire in response to danger. The MDTB consists of an oval runway based on that used in the Fear Defense Test Battery with rats [13]. However, specific situational and behavioral components of the Anxiety Defense Test Battery, involving reactivity to stimuli associated with potential threat rather than to the actual presence of an approaching predator, are incorporated into the mouse battery. Briefly, the MDTB consists of five tests either associated with potential threat (contextual defense) or the actual presence of an approaching threat (i.e. a rat). These latter focus on changes in flight, risk assessment and defensive threat/attack behaviors, while the former involves escape attempt responses from the runway cage. Drug experiments demonstrated that anxiolytic compounds generally tend to decrease defensive behaviors. However, it is noteworthy that some responses are specifically or mainly affected by certain drug classes [14,37]. Thus, BZs decrease risk assessment activities of animals chased by the rat and defensive threat and attack responses, while 5-HT_{1A} agents mainly affects contextual defense and defensive threat and attack behaviors. In addition, 5-HT reuptake inhibitors and CCK_B antagonists have a clearer impact on flight responses than on other defensive reactions. Taken together, these observations suggest that risk assessment, flight, defensive threat/attack and escape attempts probably reflect different aspects of anxiety-related reactions.

A major concern with traditional animal models of 'state' anxiety based on single (mostly spatiotemporal) measures is that they are in most cases unable to discriminate between anxiolysis induced by different classes of anxiolytics (BZs, 5-HT_{1A} agonists, 5-HT reuptake inhibitors), although clinical findings strongly indicate differential therapeutic efficacy of these agents, according to the anxiety disorder treated. Based on these observations, it is clear that the major advantage of the ethological plus-maze and the MDTB is that they provide models capable of responding to and differentiating anxiolytic drugs of different classes through specific profiles of effect on different measures. This represents a significant improvement over other animal models for evaluating drugs effective against emotional disorders.

5.2. Mouse models of 'pathological' or 'trait' anxiety

A review of the literature indicates that nearly thirty new strains of mice have been generated by using gene targeting technology which display a phenotype consistent with increased anxiety (Table 2). While a few of these phenotypes appear to reflect the known function of the target (e.g. 5-HT_{1A} receptor, corticotropin-releas-

Table 2
Mouse models of 'pathological' anxiety based on the use of gene targeting technology

Gene	Tg or KO	Genetic background	Models	Reference
α_3 Neural nicotinic acetylcholine receptor	KO	Mix (BALB/c, C57BL/6)	EPM	[77]
Adenosine A_{2a} receptor	KO	CD1	EPM, LD	[52]
Angiotensin II receptor	KO	Mix (129, C57BL/6)	EPM, LD	[43]
	KO	?	LD	[65]
Cholecystokinin$_B$ receptor	KO	Mix (129, C57BL/6)	EPM	[87]
Catechol-*O*-methyltransferase	KO	Mix (129, C57BL/6)	LD	[30]
Corticotropin-releasing factor	Tg	Mix (B6, SJL)	EPM, LD	[82]
	Tg	Mix (B6, SJL)	LD	[38]
Corticotropin-releasing factor binding protein	KO	C57BL/6	EPM, OF	[71]
	KO	C57BL/6	EPM, DF	[46]
Corticotropin-releasing factor$_2$ receptor	KO	Mix (129, C57BL/6)	EPM, LD, OF	[48]
	KO	Mix (129, C57BL/6)	OF	[22]
	KO	Mix (129, C57BL/6)	EPM,OF	[3]
Dopamine$_4$ receptor	KO	Mix (129, C57BL/6)	OF	[26]
Endothelial nitric oxide synthase	KO	C57BL/6	EPM, OF	[28]
Estrogen receptor alpha	KO	Mix (129, C57BL/6)	LD	[64]
Fyn protooncogene	KO	Mix (129, C57BL/6)	EPM, LD, OF	[61]
GABAa receptor γ_2	KO	Mix (129, C57BL/6)	EPM, LD, FE	[24]
	KO	Mix (129, C57BL/6)	EPM	[42]
Glutamic acid decarboxylase	KO	C57BL/6	EPM	[47]
	KO	?	LD	[83]
5-hydroxytryptamine$_{1a}$ receptor	KO	Mix (129, C57BL/6)	EPM	[39]
	KO	129	EPM	[70]
	KO	Mix (C57BL/6, Swiss)	OF	[68]
	KO	129	EPM	[69]
Interferon γ	KO	Mix (129, C57BL/6)	EPM	[51]
Interleukin 6	KO	Mix (129, C57BL/6)	EPM	[2]
MAS oncogene	KO	Mix (129, C57BL/6)	EPM	[88]
Midkine	KO	129	EPM	[62]
Neural cell adhesion molecule	KO	Mix (129, C57BL/6)	LD	[83]
Neuropeptide Y	Tg	Mix (C57BL/6, DBA/2)	EPM	[44]
	KO	Mix (129, C57BL/6)	EPM	[67]
	KO	?	AS, EPM	[4]
Orphanin FQ	KO	Mix (129, C57BL/6)	EPM, OF, LD	[50]
Preproenkephalin	KO	Mix (129, CD1)	EPM	[49]
Puromycin-sensitive aminopeptidase	KO	BALB/c	EPM	[66]
Single-minded 2	Tg	?	EPM	[20]
Tumor necrosis factor-α	Tg	Mix (C57BL/6, CBA)	LD	[27]

Abbreviations used: AS, acoustic startle; DF, defensive withdrawal; EPM, elevated plus-maze; FE, free-exploration; LD, light/dark; OF, open-field; SIH, stress-induced hyperthermia; KO, knock-out; tg, transgenic mice overexpressing a target protein

ing factor (CRF), neuropeptide Y) in emotional processes, many others include genes that have not been shown to be involved in anxiety behaviors earlier (e.g. fyn protooncogene, MAS oncogene, tumor necrosis factor-α).

In 1998, Ramboz and colleagues claimed that mice lacking the 5-HT_{1A} receptor by homologous recombination may represent a valid animal model of anxiety-related disorder since they showed increased emotionality in the elevated plus-maze test [70]. This finding was confirmed by several other studies which demonstrated that knockout mice lacking the 5-HT_{1A} receptors display increases in fear-related behaviors in the elevated plus-maze and several other procedures (open-field, stress-induced hyperthermia) [39,68,69]. CRF has also been largely the focus of gene targeting in order to generate animals that show increased anxiety, and thus may provide a 'pathological' model of anxiety. For example, it was demonstrated that CRF transgenic mouse lines overexpressing CRF exhibited a behavioral state resembling that produced by anxiety in the elevated plus-maze and the light/dark tests [82]. Moreover, three recent studies reported that male, but not female, CRF_2 receptor-deficient mice exhibit enhanced anxious behavior in several tests of anxiety, including the elevated plus-maze, the light/dark and the open-field tests [3,22,48]. Finally, CRF-binding protein-deficient mice were shown to exhibit a significant increase in a fear-related behavior in the elevated plus-maze, open-field and defensive withdrawal tests [46,71]. The well acknowledged involvement of GABA in the regulation of emotional processes and the anxiety-modulating action of

BZ receptor ligands, has led to the development of mice displaying a deficit in the γ_2 subunit of the $GABA_A$ receptor, which has been shown to be essential in mediating the modulatory actions of BZs. Behavioral observations showed that these mice exhibit anxiogenic-like responses in several models based on the exploration of aversive areas [23,42]. Studies using pharmacological tools have shown that the neuroactive peptide NPY may play a critical role in anxiety. Indeed, the central infusion of the neuroactive peptide NPY and NPY fragments selective for the Y_1 receptor was demonstrated to elicit anxiolytic-like effects in a variety of tests, whereas the local application of Y_1 receptor antagonists produce the opposite action [32]. On the basis of these findings, several research groups created mutant mice lacking the gene for NPY [4,67]. Data from behavioral tests revealed that these mice have an anxiogenic-like phenotype.

These genetic animal models of anxiety have at first glance clear advantages over 'state' anxiety models in which baseline levels of anxiety of a 'normal' subject are increased artificially by exposure to aversive stimuli. They may provide a unique opportunity to study human anxiety and emotional disorders. Unfortunately, all these genetic models are based on the deletion of a single gene, and it is now clear that the modulation anxiety processes involves multiple genes. There is no doubt for example that the $5\text{-}HT_{1A}$ receptor plays a role in anxiety, but it is excessive to describe mice lacking this receptor as 'an animal model of anxiety-related disorder' [70] since it is by far not the only target involved in the regulation of emotional processes. Another problem with these mouse models of 'pathological' anxiety is that the measure of anxiety has been performed in a few tests of anxiety only. Among the 38 references listed in Table 2, 27 (i.e. 71%) used the elevated plus-maze, and 35 (i.e. 92%) employed exploration tests only. In view of the above concern regarding the elevated plus-maze, experiments with mutant mice in this test require extreme caution when interpreting the data. It is possible that in a few instances, responses exhibited by these mice may relate to behavioral processes unrelated to anxiety. Finally, most of the mutant mice studies have been undertaken using only one genetic background, usually a mixed C57Bl/6 and 129 F2 strain. It would be useful to undertake behavioral studies using more then one strain, for example including a strain exhibiting a high emotionality level and a strain displaying a low emotionality. Indeed, strain differences in emotionality have repeatedly been reported (see next paragraph). Furthermore, most mutant studies used embryonic stem cells from a 129 substrain, and then cross chimaeric animals with C57Bl/6 mice. Homozygous for the targeted mutation are then generated by producing an F2 intercross with a part of the genetic background of 129 and another part of C57Bl/6. In some cases, mutant animals are then backcrossed to 129 or C57Bl/6 for two to five generations, rarely more. Unfortunately, some confounding effects may be related to the 129 substrain genes flanking the target locus which are present in the mutant animal and not in the corresponding wildtype mice (see Gerlai [29] for further details).

The use of strains of mice displaying spontaneously elevated emotionality or mice selected for their high levels of anxiety may circumvent some of the problems encountered with the above-mentioned mutant mouse models. Such animals would exhibit increased anxiety not because of the deletion of a single gene, but because it is an enduring feature of a strain or an individual, probably involving multiple genetic and environmental factors. While several animal models of 'trait' anxiety have been described in rats (e.g. Wistar–Kyoto [31], Roman line [18], Sardinian alcohol-preferring [21]), there is only one mouse strain that has shown consistently higher levels of anxiety when compared with other strains, namely the BALB/c line. For example, Makino et al. [56] demonstrated that BALB/c mice showed strong and long-lasting stretching immediately after their introduction into the open-field, while C57BL/6 and DBA/2 mice never displayed such behavior. Instead, they immediately started to move around. These authors interpreted their findings in terms of 'emotional arousal', with the BALB/c strain being more 'anxious' than the two other lines. Moreover, using several tasks based on exploratory behavior (e.g. the light/dark choice test) we confirmed that BALB/c generally show a more pronounced reluctance to locomote in a novel area than do other inbred (C57BL/6, C3H, CBA, DBA/2, NZB, SJL) and/or outbred (NMRI, Swiss) strains of mice [10,34]. Interestingly, unlike the other strains, BALB/c mice exhibit strong neophobic reactions when confronted simultaneously with a familiar and a novel compartment in the free-exploration test [33]. Based on the finding that no neurovegetative changes were apparent in mice that had free access to novelty when compared with the modifications induced by situations in which these animals were forced, the free-exploration test can be considered to be devoid of clear anxiogenic stimuli [60]. Consequently, the observation that BALB/c mice display strong neophobic reactions in this procedure indicates that neophobia represents a constant feature of their behavior. The reasons for the differences in the level of fearfulness between BALB/c mice and the other strains remain largely unknown, but certainly include many factors such as life history, test situation or housing conditions. More importantly, these differences may be due to neuroanatomical, neurochemical or genetic factors. For example, it was reported that BALB/c and C57BL/6 mice differ in the density and/or the affinity of BZ receptors [19,72]. These authors showed that the affinity

for BZ receptors is higher in BALB/c than in C57BL/6 mice, whereas the latter strain displays a greater density in BZ receptor sites than the former. However, this particularity is not limited to the BZ receptors. Indeed, electric footshock induces a higher increase in dopaminergic turnover in the prefrontal cortex of BALB/c than in C57Bl/6 mice [40]. Finally, this strain also exhibits some particular features in the sensitivity to anxiolytic agents. Indeed, it has a high sensitivity to the anxiolytic action of BZs [34] and low doses of the BZ receptor antagonist flumazenil induce an anxiolytic-like action in this strain [9]. Furthermore, naloxone, an opioid antagonist, blocks the anxiolytic-like action of BZs in SWISS and C57Bl/6 mice, but not in BALB/c mice, an effect probably related to abnormality in κ-opioidergic receptors [1,5,8]. These strain differences in the action of pharmacological agents also appear for measures not related to anxiety. For example, when compared with C57Bl/6 mice, the BALB/c strain is very sensitive to the convulsant action of the BZ inverse agonist β-CCM [24]. In the conditioned place preference test, a model relevant for the study of the subjective properties of drugs, amphetamine, a psychostimulant, produced a positive reinforcing effects in C57Bl/6 mice, while the opposite was observed in BALB/c mice [7]. Taken as a whole, these findings with BALB/c mice strongly suggest that this strain may be considered as a realistic model of 'trait' anxiety, which is not only related to one particular target (as observed in targeted mutations), but to abnormalities in various neurotransmitter systems (GABAergic, dopaminergic, opioidergic, etc.).

In conclusion, while animal models of 'state' anxiety remain the mainstay of tests used in studies dealing with emotional processes, models of 'pathological' anxiety, which are in great part based on the use of gene targeting technology, are used increasingly. However, their usefulness as models of anxiety is limited since they are based on the deletion of a single gene, which alone can hardly account for a complex condition such as anxiety. Possibly, the use of inbred 'anxious' mouse strains, which show constant high levels of fearfulness, may provide models of anxiety that have greater face, construct and/or predictive validity than 'state' or single-gene deletion models of anxiety.

References

[1] Agmo A, Belzung C, Deloire X, Grassin M, Lewis S. Blockade of anxiolytic-like actions of chlordiazepoxide by naloxone in the elevated plus-maze: comparisons between Swiss, C57BL/6 and BALB/c mice. Psychobiology 1999;27:105–13.

[2] Armario A, Hernandez J, Bluethmann H, Hidalgo J. IL-6 deficiency leads to increased emotionality in mice: evidence in transgenic mice carrying a null mutation for IL-6. J Neuroimmunol 1998;92:160–9.

[3] Bale TL, Contarino AB, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee KF. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat Genet 2000;24:410–4.

[4] Bannon AW, Seda J, Carmouche M, Francis JM, Norman MH, Karbon B, McCaleb ML. Behavioral characterization of neuropeptide Y knockout mice. Brain Res 2000;868:79–87.

[5] Belzung C, Agmo A. Naloxone blocks anxiolytic-like effects of benzodiazepines in Swiss but not in BALB/c mice. Psychopharmacology 1997;132:195–201.

[6] Belzung C, Agmo A. Naloxone potentiates the effects of subeffective doses of anxiolytic agents in mice. Eur J Pharmacol 1997;323:133–6.

[7] Belzung C, Barreau S. Differences in drug-induced place conditioning between BALB/c and C57Bl/6 mice. Pharmacol Biochem Behav 2000;65:419–23.

[8] Belzung C, Dubreuil D. Naloxone potentiates the anxiolytic but not the amnestic action of chlordiazepoxide in C57BL/6 mice. Behav Pharmacol 1998;9:691–8.

[9] Belzung C, Leguisquet AM, Crestani F. Flumazenil induces benzodiazepine partial agonist-like effects in BALB/c but not C57BL/6 mice. Psychopharmacology 2000;148:24–32.

[10] Beuzen A, Belzung C. Link between emotional memory and anxiety states: a study by principal component analysis. Physiol Behav 1995;58:111–8.

[11] Bhattacharya SK, Acharya SB. Further investigations on the anxiogenic action of isatin. Biogenic Amines 1993;9:5–6.

[12] Blanchard RJ, Blanchard DC. Affect and aggression: an animal model applied to human behavior. In: Blanchard RJ, Blanchard DC, editors. Advances in the study of aggression. Orlando: Academic Press, 1984:1–62.

[13] Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM. The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev 1990;14:463–72.

[14] Blanchard RJ, Griebel G, Henrie JA, Blanchard DC. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci Biobehav Rev 1997;21:783–9.

[15] Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci USA 1998;95:5335–40.

[16] Canguilhelm G. Le normal et le pathologique. Paris: PUF, 1999.

[17] Calatayud F, Belzung C. Emotional reactivity in mice: a case of non-genetic heredity? Physiol Behav, 2001;in press.

[18] Chaouloff F, Castanon N, Mormede P. Paradoxical differences in animal models of anxiety among the Roman rat lines. Neurosci Lett 1994;182:217–21.

[19] Chapouthier G, Bondoux D, Martin B, Desforges C, Launay JM. Genetic difference in sensitivity to beta-carboline: evidence for the involvement of brain benzodiazepine receptors. Brain Res 1991;553:342–6.

[20] Chrast R, Scott HS, Madani R, Huber L, Wolfer DP, Prinz M, Aguzzi A, Lipp HP, Antonarakis SE. Mice trisomic for a bacterial artificial chromosome with the single-minded 2 gene (SIM2) show phenotypes similar to some of those present in the partial trisomy 16 mouse models of down syndrome. Hum Mol Genet 2000;9:1853–64.

[21] Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, Gessa GL. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. Physiol Behav 1995;57:1181–5.

[22] Coste SC, Kesterson RA, Heldwein KA, Stevens SL, Heard AD, Hollis JH, Murray SE, Hill JK, Pantely GA, Hohimer AR, Hatton DC, Phillips TJ, Finn DA, Low MJ, Rittenberg MB, Stenzel P, StenzelPoore MP. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. Nat Genet 2000;24:403–9.

[23] Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Luscher B, Mohler H. Decreased $GABA_A$-receptor clustering results in enhanced anxiety and a bias for threat cues. Nat Neurosci 1999;2:833–9.

[24] Desforges C, Venault P, Dodd RH, Chapouthier G, Roubertoux PL. Carboline-induced seizures in mice: genetic analysis. Pharmacol Biochem Behav 1989;34:733–7.

[25] DSM-IV. Diagnostic and Statistical Manual of Mental Disorders, Fourth ed. Washington, DC: American Psychiatric Association, 1994.

[26] Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J Neurosci 1999;19:9550–6.

[27] Fiore M, Alleva E, Probert L, Kollias G, Angelucci F, Aloe L. Exploratory and displacement behavior in transgenic mice expressing high levels of brain TNF-alpha. Physiol Behav 1998;63:571–6.

[28] Frisch C, Dere E, Silva MAD, Godecke A, Schrader R, Huston JP. Superior water maze performance and increase in fear-related behavior in the endothelial nitric oxide synthase-deficient mouse together with monoamine changes in cerebellum and ventral striatum. J Neurosci 2000;20:6694–700.

[29] Gerlai R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci 1996;19:177–81.

[30] Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M. Catechol-*O*-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci USA 1998;95:9991–6.

[31] Goto SH, Conceicao IM, Ribeiro RA, Frussa Filho R. Comparison of anxiety measured in the elevated plus-maze, open-field and social interaction tests between spontaneously hypertensive rats and Wistar EPM-1 rats. Braz J Med Biol Res 1993;26:965–9.

[32] Griebel G. Is there a future for neuropeptide receptor ligands in the treatment of anxiety disorders? Pharmacol Ther 1999;82:1–61.

[33] Griebel G, Belzung C, Misslin R, Vogel E. The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs. Behav Pharmacol 1993;4:637–44.

[34] Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology 2000;148:164–70.

[35] Griebel G, Blanchard DC, Jung A, Blanchard RJ. A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. Behav Pharmacol 1995;6:732–45.

[36] Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 1995;65:319–95.

[37] Griebel G, Sanger DJ. The mouse defense test battery: an experimental model of different emotional states. In: Haug M, Whalen RE, editors. Animal Models of Human Emotion and Cognition. Washington, DC: American Psychological Association, 1999:75–85.

[38] Heinrichs SC, Min H, Tamraz S, Carmouche M, Bochme SA, Vale WW. Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. Psychoneuroendocrinology 1997;22:215–24.

[39] Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, Tecott LH. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Nat Acad Sci USA 1998;95:15049–54.

[40] Herve D, Tassin JP, Barthelemy C, Blanc G, Lavielle S, Glowinski J. Difference in the reactivity of the mesocortical dopaminergic neurons to stress in the BALB/c and C57 BL/6 mice. Life Sci 1979;25:1659–64.

[41] Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 1996;54:21–30.

[42] Homanics GE, Harrison NL, Quinlan JJ, Krasowski MD, Rick CE, De Blas AL, Mehta AK, Kist F, Mihalek RM, Aul JJ, Firestone LL. Normal electrophysiological and behavioral responses to ethanol in mice lacking the long splice variant of the γ2 subunit of the γ-aminobutyrate type A receptor. Neuropharmalogy 1999;38:253–65.

[43] Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BL, Inagami T. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. Nature 1995;377:748–50.

[44] Inui A, Okita M, Nakajima M, Momose K, Ueno N, Teranishi A, Miura M, Hirosue Y, Sano K, Sato M, Watanabe M, Sakai T, Watanabe T, Ishida K, Silver J, Baba S, Kasuga M. Anxiety-like behavior in transgenic mice with brain expression of neuropeptide Y. Proc Assoc Am Physicians 1998;110:171–82.

[45] Kaplan A. The Conduct of Inquiry: Methodology for Behavioral Sciences. Aylesbury, Buckinghamshire: International Textbook Company, 1973.

[46] Karolyi IJ, Burrows HL, Ramesh TM, Nakajima M, Lesh JS, Seong E, Camper SA, Seasholtz AF. Altered anxiety and weight gain in corticotropin-releasing hormone-binding protein-deficient mice. Proc Nat Acad Sci USA 1999;96:11595–600.

[47] Kash SF, Tecott LH, Hodge C, Baekkeskov S. Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc Nat Acad Sci USA 1999;96:1698–703.

[48] Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J. Deletion of Crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nat Genet 2000;24:415–9.

[49] Konig M, Zimmer AM, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, Zimmer A. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 1996;383:535–8.

[50] Köster A, Montkowski A, Schulz S, Stube EM, Knaudt K, Jenck F, Moreau JL, Nothacker HP, Civelli O, Reinscheid RK. Targeted disruption of the orphanin FQ/nociceptin gene increases stress susceptibility and impairs stress adaptation in mice. Proc Nat Acad Sci USA 1999;96:10444–9.

[51] Kustova Y, Sei Y, Morse HC Jr., Basile AS. The influence of a targeted deletion of the IFNgamma gene on emotional behaviors. Brain Behav Immun 1998;12:308–24.

[52] Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature 1997;388:674–8.

[53] Lee C, Rodgers RJ. Effects of buspirone on antinociceptive and behavioural responses to the elevated plus-maze in mice. Behav Pharmacol 1991;2:491–6.

[54] Lister RG. Ethologically-based animal models of anxiety disorders. Pharmacol Ther 1990;46:321–40.

[55] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.

[56] Makino J, Kato K, Maes FW. Temporal structure of open-field behavior in inbred strains of mice. Jpn Psychol Res 1991;33:145–52.

[57] McKinney WT. Animal models of depression: an overview. Psychiatr Dev 1984;2:77–96.

[58] McKinney WT Jr., Bunney WE Jr. Animal model of depression. I. Review of evidence: implications for research. Arch Gen Psychiatry 1969;21:240–8.

[59] Micheau J, vanMarrewijk B. Stimulation of 5-HT1A receptors by systemic or medial septum injection induces anxiogenic-like effects and facilitates acquisition of a spatial discrimination task in mice. Prog Neuro Psych Biol Psych 1999;23:1113–33.
[60] Misslin R, Cigrang M. Does neophobia necessarily imply fear or anxiety? Behavioural Processes 1986;12:45–50.
[61] Miyakawa T, Yagi T, Watanabe S, Niki H. Increased fearfulness of Fyn tyrosine kinase deficient mice. Brain Res Mol Brain Res 1994;27:179–82.
[62] Nakamura E, Kadomatsu K, Yuasa S, Muramatsu H, Mamiya T, Nabeshima T, Fan QW, Ishiguro K, Igakura T, Matsubara S, Kaname T, Horiba M, Saito H, Muramatsu T. Disruption of the midkine gene (MDK) resulted in altered expression of a calcium binding protein in the hippocampus of infant mice and their abnormal behaviour. Genes Cells 1998;3:811–22.
[63] Nunes de Souza RL, CantodeSouza A, DaCosta M, Fornari RV, Graeff FG, Pela IR. Anxiety-induced antinociception in mice: effects of systemic and intra-amygdala administration of 8-OH-DPAT and midazolam. Psychopharmacology 2000;150:300–10.
[64] Ogawa S, Lubahn DB, Korach KS, Pfaff DW. Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci USA 1997;94:1476–81.
[65] Okuyama S, Sakagawa T, Chaki S, Imagawa Y, Ichiki T, Inagami T. Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor. Brain Res 1999;821:150–9.
[66] Osada T, Ikegami S, TakiguchiHayashi K, Yamazaki Y, Katoh-Fukui Y, Higashinakagawa T, Sakaki Y, Takeuchi T. Increased anxiety and impaired pain response in puromycin-sensitive aminopeptidase gene-deficient mice obtained by a mouse gene-trap method. J Neurosci 1999;19:6068–78.
[67] Palmiter RD, Erickson JC, Hollopeter G, Baraban SC, Schwartz MW. Life without neuropeptide Y. Recent Prog Horm Res 1998;53:163–99.
[68] Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA 1998;95:10734–9.
[69] Pattij T, Sarnyai Z, Brunner D, Olivier B. Does the 5-HT1A receptor knockout mouse have an anxious phenotype? Int J Psychopharmacol 2000;3(Suppl. 1):S275.
[70] Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 1998;95:14476–81.
[71] Ramesh TM, Karolyi IJ, Nakajima M, Camper SA, Seasholtz AF. Altered physiological and behavioral responses in CRH-binding protein deficient mice. Soc Neurosci Abstr 1998;24:505–5.
[72] Robertson HA. Benzodiazepine receptors in 'emotional' and 'non emotional' mice: comparison of four strains. Eur J Pharmacol 1979;56:163–6.
[73] Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997;30:289–304.
[74] Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, editors. Ethology and Psychopharmacology. Chichester: Wiley, 1994:9–44.
[75] Rodgers RJ, Cole JC, Cobain MR, Daly P. Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: Profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behav Pharmacol 1992;3:621–34.
[76] Rodgers RJ, Johnson NJT. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol Biochem Behav 1995;52:297–303.
[77] Ross SA, Wong JYF, Clifford JJ, Kinsella A, Massalas JS, Horne MK, Scheffer IE, Kola I, Waddington JL, Berkovic SF, Drago J. Phenotypic characterization of an alpha(4) neuronal nicotinic acetylcholine receptor subunit knock-out mouse. J Neurosci 2000;20:6431–41.
[78] Rupniak NM, Kramer MS. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. Trends Pharmacol Sci 1999;20:485–90.
[79] Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B. Animal models of anxiety and the development of novel anxiolytic drugs. Prog Neuro Psychopharmacol Biol Psychiatry 1991;15:205–12.
[80] Seale TW, Niekrasz I, Garrett KM. Anxiolysis by ethanol, diazepam and buspirone in a novel murine behavioral assay. NeuroReport 1996;7:1803–8.
[81] Skinner, B.F., Contingencies of Reinforcement: a theoretical analysis, Appleton-Century-Crofts, New-York, 1969.
[82] Stenzel-Poore MP, Duncan JE, Rittenberg MB, Bakke AC, Heinrichs SC. CRH overproduction in transgenic mice: behavioral and immune system modulation. Ann NY Acad Sci 1996;780:36–48.
[83] Stork O, Welzl H, Wotjak CT, Hoyer D, Delling M, Cremer H, Schachner M. Anxiety and increased 5-HT1A receptor response in NCAM null mutant mice. J Neurobiol 1999;40:343–55.
[84] Treit D. Animal models for the study of anti-anxiety agents: a review. Neurosci Biobehav Rev 1985;9:203–22.
[85] Tsang CF, Wilkinson GR. Diazepam disposition in mature and aged rabbits and rats. Drug Metab Dispos 1982;10:413–6.
[86] Tweed JL, Schoenbach VJ, George LK, Blazer DG. The effects of childhood parental death and divorce on six-month history of anxiety disorders. Br J Psychiatry 1989;154:823–8.
[87] Vasar E, Koks S, Beljajev S, Abramov U, Koovit I, Matsui T. CCKB receptor knockout mice: gender related behavioural differences. Eur Neuropsychopharmacol 2000;10(Suppl. 2):S69.
[88] Walther T, Balschun D, Voigt JP, Fink H, Zuschratter W, Birchmeier C, Ganten D, Bader M. Sustained long term potentiation and anxiety in mice lacking the Mas protooncogene. J Biol Chem 1998;273:11867–73.
[89] Weiss SM, Lightowler S, Stanhope KJ, Kennett GA, Dourish CT. Measurement of anxiety in transgenic mice. Rev Neurosci 2000;11:59–74.
[90] Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 1992;16:525–34.